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PRINCIPAL INVESTIGATOR: Craig C. Malbon, Ph.D.

CONTRACTING ORGANIZATION: The Research Foundation of SUNY
Stony Brook, NY 11794-3362

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Craig C. Malbon, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)The Research Foundation of SUNY
Stony Brook, NY 11794-3362

E-Mail: craig@pharm.som.sunysb.edu

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During the last FY of the award, the Cancer Institute of Long Island benefited from CPMRP funding in a manner consistent with the proposed activities of the award. In the area of core instrumentation acquisition, the Applied Biosystems Inc. Q-Star LC/MS/MS Mass Spectrometer that was acquired in year-01 became fully operational and has supported research for twenty-one cancer investigators from seven different departments. Data for several publications and new grants are being organized and the instrumentation is a widely accepted successful addition to the Cancer Proteomics initiative. Additionally another research grade ABI MALDI-ToF was acquired, as well as preparatory robots to aid in spot excision and gel digestion. More recently, as specified in year-02 much time was spent investigating and considering the best option for a 2-photon confocal microscope. Upon the conclusion of this process a Carl Zeiss Inc. Inverted Multi-Photon system was selected. The instrument is to be sited and operated in a class 100,000 clean room and located in a shared instrument facility contiguous to aspect of the cancer marker diagnostics laboratory as well as many other cancer research groups. The technology offers a new and exciting platform for cancer imaging and has been equipped to enable the imaging of living cells. Leading this aspect is the thematic program director, and now chair of the Department of Molecular Genetics & Microbiology, Dr. Dafna Bar-Sagi. A faculty member skilled in 2-Photon Microscopy will provide the daily oversight for the instrument, and as well a PhD level optical physicist will be recruited to operate the instrument. The instrument will be delivered and installed in September of 2003.

Consistent with the funding for this initiative were the granting of RSU packages, or Research Support Units. These units serve to assist in the recruitment of new, well-trained cancer researchers. Each package provided support for laboratory personnel, small or specialized research equipment, and supplies. During the last funding year two such individuals were recruited into this initiative. The first, Dr. Howard Adler, MD, Assistant Professor of Urology was provided support under this mechanism. Dr. Adler is a translational researcher, trained at Baylor University, who is investigating the angiogenesis of prostate cancer. His research mentor is Dr. Stanley Zucker, MD, Professor of Medical-Oncology. The second individual is Dr. Howard Crawford, PhD, Assistant Professor of Pharmacological Sciences. Dr. Crawford is a cancer protein and cell-signaling researcher, trained at Vanderbilt University. His mentor is Dr. Jeffrey Pessin, PhD, Professor and Chair of Pharmacological Sciences. Dr. Crawford's expertise in pancreatic and breast cancer.

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Introduction:

The State University of New York at Stony Brook, School of Medicine continues to develop its infrastructure for to support a Comprehensive Cancer Center in central Long Island, New York. Achievements vital to this year of CDMRP funding are summarized below. CDMRP funding focused in cancer research infrastructure has greatly assisted the efforts of the School of Medicine by enabling our ability to provide a foundation for aspiring young scientists. Drs. Adler and Crawford were recently recruited to Stony Brook. Both have promising careers as academic cancer researchers. Dr. Adler is a member of the clinical faculty in the Dept. of Urology. Dr. Crawford is a member of the basic sciences faculty in the Dept. of Pharmacology. Additionally, CDMRP funds allocated towards core technologies have enabled the School of Medicine to secure and bring on-line two new state-of-the-art instruments for cancer research. A Carl Zeiss Microsystems Inc. Multi-Photon Imaging System, and an Applied Biosystems Q-Star Pulsar I LC/MS/MS instrument are now (or are nearly) available for faculty access. Both of these instruments are available as cancer research center core instruments and are operated by skilled technicians. The instruments are sited centrally, and are supported by ancillary equipment made available by the School of Medicine via other funding sources.

Body:**1) Cancer Invasion & Angiogenesis - Dr. Howard Adler, MD, Assistant Professor of Urology:**

Evaluating the Role of Matrix Metalloproteinases in Prostate Cancer Progression- Working in collaboration with Drs. Stan Zucker (Research Mentor) and Jian Cio. The goal of this project is to examine the role of membrane type 1-matrix metalloproteinase in prostate cancer progression and metastasis by establishing a stable LNCaP (an androgen dependent cell line which does not produce endogenous type-1 matrix metalloproteinase, MT1-MMP) cell lines expressing MT1-MMP/GFP chimera and a GFP control. The following steps have been completed:

- 1) 2×10^6 stable cells expressing GFP and MT1/GFP were injected orthotopically into 4-week old NCI/nu male mice. Each group contained 10 mice
- 2) Five mice (2 from GFP group and 3 from MT1/GFP group) died the next day.
- 3) Weight changes: a) A week later after prostate injection (2-11-03), weight gain from the MT1/GFP group was slower than that of the GFP group. The mean of weight gain for GFP group was 4.11g, while the mean of weight gain for MT1/GFP group was 3.72. This result is consistent with the weight gain from subcutaneous tumor injection groups. a) The weight loss from both groups began week 8 after injection until sacrifice at week 11 (all mice were sacrificed at week 11).
- 4) GFP group: a) Tumorigenesis: two out of eight mice had prostate tumor; four mice had tumor but outside the prostate; no tumor was found in two other mice. b) All tumor has green fluorescence under UV light indicating expression of GFP/LNCaP cells. c) No visible enlarged lymph nodes were found. No metastatic cells was found under fluorescent microscopy, d) No tumor nodules were found on the surface of lung, but most of the mice had micro-metastasis (a single cell or a few cells) in the lung under fluorescent microscopy; This data suggests LNCaP cells orthotopically injected into the prostate can undergo metastasis to the lung, but determination of lung metastasis

needs a sensitive approach, such as the GFP marker to detect it (previous reports denied tumor metastasis of LNCaP cells). e) No metastatic cells in liver and kidney. f) Sections from different organs are under examination.

- 5) MT1/GFP group: a) Tumorigenesis: three out of 7 had tumor growth, but one in an early stage. b) All tumor had green fluorescence under UV light. c) Lymph nodes from tumor bearing mice were, but no metastatic tumor cells (GFP fluorescence) were found. d) No tumor nodules were found on the surface of lung, but large tumor emboli and metastatic tumors were found in the lung. Compared to metastatic tumor in the lung from GFP group, there were more metastatic tumors in MT1/GFP group. e) No metastatic cells in liver and kidney. f) Sections from different organs are under examination.

The plan is to increase experimental groups for high, medium and low expression of MT1-GFP/LNCaP. It is expected that repeat experiments will lead to better results as experience with establishing orthotopic tumors increases. The CDMRP support to for this project allowed Dr. Adler to obtain the protected time needed to achieve his academic goals. He has been able to attend and participate in the weekly lab meeting held in Dr. Zucker's laboratory at the Northport VA. This opportunity has allowed Dr. Adler to not only improve his abilities to critically evaluate research at the basic science level, but to find more opportunities to expand into the basic science research realm. Specifically, as he is able to provide investigators with orthotopic prostate cancer models, he has been able to assist Dr. Gayle Vaday, of SUNY-Stony Brook with a grant submission to CaPCure that seeks to evaluate the chemokine regulation of prostate cancer metastasis and investigate potential new therapeutic modalities for the treatment of prostate cancer. The orthotopic tumor model is a crucial part to Dr. Vaday's research. In time, we are hopeful that the strong relationship that Dr. Adler has developed with the basic science investigators at Stony Brook and the VA alike will lead to successful research in the field of prostate cancer.

2) Recruitment of Dr. Howard Crawford, Ph.D., Assistant Professor Pharmacological Sciences:

Signal Transduction – Matrix Metalloproteinase-7

Matrix metalloproteinase-7 (MMP-7, matrilysin) has been shown to contribute to both the formation and invasion of adenocarcinomas in several glandular tissues. MMP-7 expression is highly restricted in normal tissue, but is frequently found in tumor cells of benign and well-differentiated invasive tumors of the breast, intestine, prostate, esophagus, stomach and pancreas. Recently, my laboratory has focused on the role of MMP-7 in pancreatic cancer, the 5th most common cause of cancer-related death in the United States. We have found that MMP-7 is expressed by the tumor cells in 98% of pancreatic ductal adenocarcinoma (PDAC) patient samples examined, by far exceeding the frequency of MMP-7 expression in tumors of other tissues. Expression ranged from the earliest stages of tumor formation through to invasive carcinoma. Strikingly, MMP-7 was also expressed by metaplastic duct epithelium in 100% of PDAC samples examined. Metaplastic duct epithelium, particularly that formed in the context of chronic pancreatitis (CP), has been hypothesized to act as a tumor precursor. With this in mind, we found that MMP-7 is expressed in the metaplastic ducts of 93% of CP samples. Most importantly, by inducing CP in mice that have had the MMP-7 locus inactivated by homologous recombination (MMP-7^{-/-}), we find that all aspects of CP are severely inhibited, including the formation of metaplastic duct epithelium.

Thus, it is surmised that MMP-7 is involved in pancreatic tumor formation through its ability to promote the formation of metaplastic duct epithelium. In this application, we propose to

systematically dissect the multiple potential roles of MMP-7 in CP and PDAC with particular emphasis on acinar-to-ductal metaplasia. We will continue to test the overall hypothesis that MMP-7, and the proteins that direct its expression to pancreatic metaplastic ductal epithelium, control the transition of normal epithelium to metaplastic, dysplastic and ultimately to neoplastic epithelia.

3) Cancer Proteomics Core Research Support –

To further support the institutional-wide effort Protein Chemistry and Analysis Laboratory has been created to support investigators committed to this initiative throughout the campus. This laboratory is located in approximately ~1,000 square feet of dedicated, newly renovated, wet-bench space on the 9th floor of the Basic Sciences Tower. Two PhD level Mass Spectroscopists, and two MS level technicians staff the facility. Services offered in this core include:

- Research Grade MALDI-ToF mass analysis via an ABI Voyager DE Star
- Research Grade LC/MS/MS mass analysis via an ABI Q-Star Pulsar I
- Analytical and Preparative HPLC
- Automated 2D Gel processing via Bio-Rad Proteome System
- Automated Protein Digestion via Perkin Elmer Multi-Probe HT
- Edman Protein Sequencing
- Ciphergen SELDI
- BiaCore Plasmon Resonance Detector

4) Cancer Imaging Core Research Support-

In keeping with instrumentation commitments outlined in the awarded application, the School of Medicine spearheaded an initiative to evaluate commercially available multi-photon, confocal imaging platforms. This initiative was led by the two newly recruited Basic Science Chairs (Drs. Pessin, Pharmacological Sciences and Bar-Sagi, Molecular Genetics & Microbiology), with guidance from the Dr. Malbon, Vice-Dean for Scientific Affairs. The selected system is an inverted Carl Zeiss Micro-Imaging product equipped with a Coherent Laser Group Chameleon Laser. A purchase order was issued in June and the instrument was delivered in October 2003. In order to support this instrument a class 100,000 modular clean enclosure is being constructed, in which the microscope will be installed, validated and operated (December 2003). An effort is underway to recruit PhD level or PE optical engineer to serve as the instrument operator. The site plan for the instrument includes the pouring of a 4" 1500 PSI concrete slab upon which the clean enclosure will be assembled. The slab will further reduce vibration in the site, thus insuring the highest possible optical image quality. Likewise, the air handlers for the enclosure will be remote located to eliminate any additional dynamic vibration. The enclosure has been designed with an interior wall height of 9'. This aspect will diminish white noise and air velocity / turbulence disturbance.

5) Creation of a Cancer Tissue Bank-

The critical, value-added element of the CDMRP award was the collateral efforts to develop an institutional Cancer Tissue Bank. The organization of commitments (totaling more than \$130,000) from the School of Medicine, University Hospital, Vice-President for Research, and the Departments of Molecular Genetics & Microbiology, Pharmacology, and Pathology leveraged an additional \$60,000 of support from the National Cancer Institute for a capital equipment supplement. The instrument selected is a Carl Zeiss Laser Capture Microdissection Microscope. Histology technicians under the supervision of a clinical pathologist will staff the bank. The Tissue Bank is located in a newly renovated, well-equipped wet laboratory of approximately 860 square feet. Cancer researchers will have full access to a trained histology staff capable of providing tissue grossing and dissection services for difficult/unique specimens

Key Research Accomplishments:

The CDMRP funding awarded to Stony Brook via this mechanism is directed towards providing infrastructure support to better serve the needs of the faculty.

Accomplishments for this reporting period include:

- 1) Instrumentation- Bring the LC/MS/MS instrument online.*
- 2) Instrumentation- Merging the University Mass Spectrometry Facility research grade MALDI-ToF, and technical support line into the Proteomics Center.**
- 3) Instrumentation- Expanding the Proteomics Center capability to include automated cancer sample preparation.**
- 4) Instrumentation- Acquisition of a Ciphergen Biosystems SELDI Instrument for Cancer Proteomics.**
- 5) Instrumentation – Acquisition of a Carl Zeiss Laser Capture Microdissection Microscope for the Cancer Tissue Bank.**
- 6) Instrumentation- Acquisition of a Carl Zeiss Multi-Photon Imaging System.*
- 7) Instrumentation- Construction of a Clean Enclosure (Cl. 100,000) to support the Multi-Photon Imager.*
- 8) Instrumentation- Equipping with a custom designed, fail-safe, research gas system to support the Multi-Photon Imager.*
- 9) Instrumentation- Recruitment of a qualified technician to operate the Multi-Photon Imager.*
- 10) Instrumentation- Expansion and updating of our Affymetrix DNA Microarray Facility instrumentation to include an additional Fluidics Station and a new GeneChip Imager.**
- 11) Instrumentation- Expansion of our DNA Microarray Facility validation systems to include an additional RT-PCR instrument (MJ Research Opticon).**
- 12) General- Securing a multi-year site license for Silicon Genetics GeneSpring.**
- 13) Faculty Development- Providing start-up funds enhancement to Dr. Adler via a mentored intramural program.*
- 14) Faculty Development- Providing start-up funds enhancement to Dr. Crawford via a mentored intramural program.*
- 15) More than 20 key publications produced by the cancer research faculty in the School of Medicine. A fully annotated citation list is available for review in the appendix of this report.

*= Benefit derivative of CDMRP funds.

**= Benefit derivative of funds allocated to complement CDMRP initiative at Stony Brook.

Reportable Outcomes:

1- *Research Support Units*- The RSU support provided to Drs. Adler and Crawford has not yet produced sufficient data to be published in peer-reviewed journals. Both Drs. Adler and Crawford are aware of the requirement to cite CDMRP support in their manuscripts.

2- *Cancer Proteomics Core*- To date this facility has provided services for cancer protein sample analysis to over twenty principal investigators, representing seven departments, and all five thematic research groups of the Cancer Institute (Cancer Invasion & Angiogenesis, Molecular Toxicology & DNA Repair, Growth Control & Differentiation, Gene Therapy, and Signal Transduction).

3- *Cancer Imaging Core*- To date the instrumentation has been received and will be installed in December 2003. Ancillary support systems are being completed within the next several days of this report. The technical-line operator will be recruited and trained in December 2003.

Conclusions:

The results of the beneficial infrastructure support that the CDMRP provides to the School of Medicine is funding of the two RSU packages for Drs. Adler and Crawford. It is anticipated that another two RSU packages will be provided during the 2003-2004 year utilizing CDMRP funding. Additionally, CDMRP funds have brought about two key advances in cancer research instrumentation for the Stony Brook faculty who participate in the Cancer Center. CDMRP capital instrumentation funds for the 2003-2004 year will again bring about additional advances in cancer research imaging, and high-throughput DNA sequencing.

References:

Not applicable. CDMRP funding is targeted towards infrastructure support.

Appendices:

23 selected publications of interest. Reprints are available upon request.

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